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RESEARCH ARTICLE

SEROPREVALANCE OF DENGUE AND CHIKUNGUNYA CO INFECTION AND ITS CLINICAL CORRELATION IN BANGALORE CITY HOSPITALS

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ABSTRACT

Introduction: Arthropod-borne viruses or arboviruses are one of the major public health problems worldwide. Out of many arboviruses, chikungunya virus (CHIKV) and dengue virus (DENV) are the two most rapidly spreading arboviruses. Serological investigations in Southern India indicate that the two viruses can co-exist in the same host. Many risk factors for chikungunya virus (CHIKV) and dengue virus (DENV) infections are the same or similar.

OBJECTIVES: The present study is conducted to know the seroprevalance of dengue and chikungunya co infection and its clinical correlation.

Materials and Methods: The blood samples collected from patients were sent along with details of the patient, clinical findings, and investigations done from various Bangalore city hospitals from the month of January 2013 to December2013, to check for the presence of IgM antibodies in serum against Dengue or Chikungunya were subjected to an enzyme-linked immunosorbent assay (ELISA) test to detect the presence of immunoglobulin M (IgM) antibodies against both CHIKV and DENV.

Results: Out of 4296 serum samples studied 205 (7.6%) samples were positive for both Dengue and Chikungunya. Majority of the cases were from the age group of 11-20 years (31.21%). A seasonal peak was seen in the months of June to August. Of the total number of affected cases, 89(43.41%) were females and 116 (56.58%) were males. Fever and was seen in almost all the cases (99 %), thrombocytopenia in 92.17%, and myalgia in 56.1% of seropositive cases. Chills, headache, arthralgia, vomiting were observed in 60.48%, 48.8 %, 40.4% and 35.6% seropositive cases respectively. Features of dengue complications like hypotension, hypoalbuminemia, rising hematocrit and hemorrhagic manifestations were seen in 7.8%, 1.4%, 2.4% and 7.3% cases respectively.

Conclusion: With the urbanization that is occurring in India, the incidence of dengue infection is increasing dramatically. With the expectation that cases of co infection with DENV and CHIKV will become more prevalent in the future due to increased transmission of both viruses in various areas of India, enhanced surveillance to clinically and diagnostically differentiate CHIKV and DENV infections is needed for early recognition of virus invasion and local transmission, better patient care, and timely control measures. With clinical examination of CHIKV/DENV coinfected patients has not yet allowed the identification of specific or severe symptoms, such observations should be interpreted with caution. Our findings may add to the recognition of CHIKV/DENV coinfections and suggest that tests to detect the presence of both viruses should be carried out in individuals showing clinical signs of an infection with either CHIKV or DENV.

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INTRODUCTION

Arthropod-borne viruses or arboviruses are one of the major public health problems worldwide. Out of many arboviruses, chikungunya virus (CHIKV) and dengue virus (DENV) are the two most rapidly spreading arboviruses. Dengue fever, caused by a flavivirus in the family Flaviviridae, is the most prevalent arboviral disease in tropical and subtropical regions of Asia, the Pacific and Caribbean islands, and Central and South America. Chikungunya, caused by an alpha virus in the family Toga viridae, is endemic to Africa and Asia.

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CHIKV and DENV are co-circulating in India and Southeast Asia (Shu-Fen Chang *et al.,* 2010). Both viruses are the RNA virus and the diseases caused by them are transmitted to humans by the vector mosquitoes Aedes aegypti and Aedes albopictus, in tropical and subtropical zones between 30° N and 40°S (Mandell, Douglas ?; Taraphdar *et al.,* 2012; Manson's Tropical Diseases 22nd edition Section 6 Chapter 41 Dengue and Dengue Haemorrhagic Fever ?). In India, Aedes aegypti mosquitoes are primary vectors for DENV and CHIKV, and opportunities for co-infections in humans are increased by the feeding behavior of the mosquitoes, low socioeconomic conditions, rapid human population growth, unplanned urbanisation, and increased international travel (Usha Kalawat *et al.,* 2011; Mohanty *et al.,* 2013).

The explosive epidemics of chikungunya in Indian Ocean islands and India since 2004 and the worldwide increase in travel have facilitated the expansion of different strains of CHIKV overlap into areas where DENV is endemic. (Shu-Fen Chang *et al.,* 2010) In India, concurrent isolation of CHIKV and DENV had been reported since 1964 from different States. In 1967, co-infections with dengue and CHIK viruses were reported from Kolkata. (Taraphdar *et al.,* 2012) Subsequent serological investigations in Southern India indicated that the two viruses can co-exist in the same host (Chahar *et al.,* 2009). The first case report of CHIK and dengue co-infection confirmed by molecular assays was from Sri Lanka In 2010, a hospital-based study revealed co-circulation of CHIKV and DENV in some areas of West Bengal, India with high morbidity (Taraphdar *et al.,* 2012).

Both diseases have some common signs and symptoms that include fever, rashes, joint pain, nausea, headache, and vomiting; Thus, many risk factors for chikungunya virus (CHIKV) and dengue virus (DENV) infections are the same or similar (Taraphdar *et al.,* 2012). Hence in areas where Dengue virus circulates, Chikungunya goes undiagnosed. A laboratory test is required to distinguish between the two diseases (Chahar *et al.,* 2009).

MATERIALS AND METHODS

The blood samples collected from patients were sent along with details of the patient, clinical findings, and investigations done. Samples received in the National Institute of Virology from various Bangalore city hospitals from the month of January 2013 to December 2013, to check for the presence of IgM antibodies in serum against Dengue or Chikungunya were included in the study (Mohanty *et al.,* 2013). All the samples were subjected to an enzyme-linked immunosorbent assay (ELISA) test to detect the presence of immunoglobulin M (IgM) antibodies against both CHIKV and DENV by IgM antibody-capture (MAC)-ELISA kits (Arbovirus Diagnostic NIV, Pune, India). The sensitivity and specificity for the CHIK IgM antibody capture ELISA is 95 % and 97.22%, respectively, and for dengue IgM antibody capture ELISA is 98.53% and 98.84%, respectively. The tests were carried out following the manufacturer's instructions (Mohanty *et al.,* 2013).

Principle of IgM Capture ELISA for CHIK and Dengue

IgM antibodies in the patient's blood are captured by anti-human IgM (μ chain specific) that are coated on to the solid surface (wells). In the next step, CHIK/Dengue antigen is added, which binds to captured IgM, if the IgM and antigen are homologous. Unbound antigen is removed during the washing step. In the subsequent steps Biotinylated anti-CHIK/Dengue monoclonal antibody is added followed by Avidin-Horse radish peroxidase (HRP). Subsequently, substrate/chromogen, Tetramethylbenzidine (TMB) - H_2O_2 is added and monitored for development of color. The reaction is stopped by $1NH_2SO_4$. The intensity of colour/optical density is measured at 450 nm. Clinical manifestations of the patients were recorded from the data available.

RESULTS

A total of 4296 serum samples from suspected cases were tested for Dengue and Chikungunya during the period from January 2013 to December 2013, out of which 205 (7.6%, Chart 1) samples were positive for both Dengue and Chikungunya. Majority of the cases were from the age group of 11-20 years (31.21%) followed by 21-35 years (27.8%, Chart 2). A seasonal peak was seen in the months of June to August [Chart 3]. Of the total number of affected cases, 89(43.41%) were females and 116 (56.58)% were males [Chart 4]. Fever and was seen in almost all the cases (99 %), thrombocytopenia in 92.17%, and myalgia in 56.1% of seropositive cases [Table 1]. Chills, headache, arthralgia, vomiting were observed in 60.48%, 48.8 %, 40.4% and 35.6% seropositive cases respectively [Table 1]. Features of dengue complications like hypotension, hypoalbuminemia, rising hematocrit and hemorrhagic manifestations were seen in 7.8%, 1.4%, 2.4% and 7.3% cases respectively [Chart 5].

Table 1.

Clinical features	Co infected Cases (%)
Fever	203 (99%)
Chills	124 (60.48%)
Myalgia/body ache	115(56.1%)
Arthralgia/difficulty in movement	82(40.4%)
Rash	14(6.82%)
Headache	100(48.8%)
Nausea/vomiting	73(35.6%)
Thrombocytopenia	189(92.17%)
Hypotension	16(7.8%)
Hypoalbuminemia	3 (1.4%)
Rising hematocrit	5 (2.4%)
Bleeding diathesis	15 (7.3%)

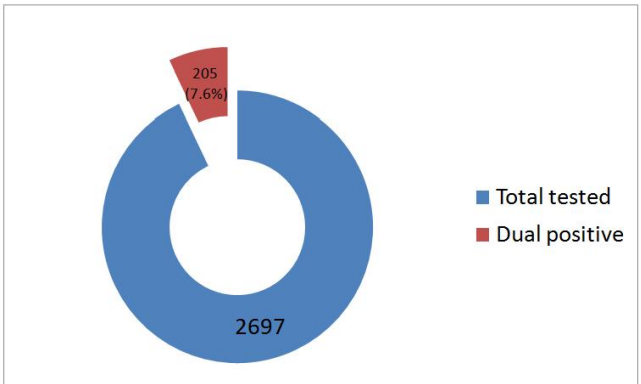

Chart 1: Total Dengue and Chikungunya IgM dual positive cases

DISCUSSION

After a quiescence of about three decades, outbreak of CHIK with sporadic cases of dengue is being reported from different parts of India. Serological investigations in Southern India indicate that the two viruses can co-exist in the same host. Co infection with DENV and CHIKV is becoming more prevalent due to increased transmission of both viruses in various areas of India. In our study only 14.6% of co infected cases were clinically suspected as Dengue and Chikungunya co infection whereas the rest (85.4%) were diagnosed clinically as either Dengue or Chikungunya.

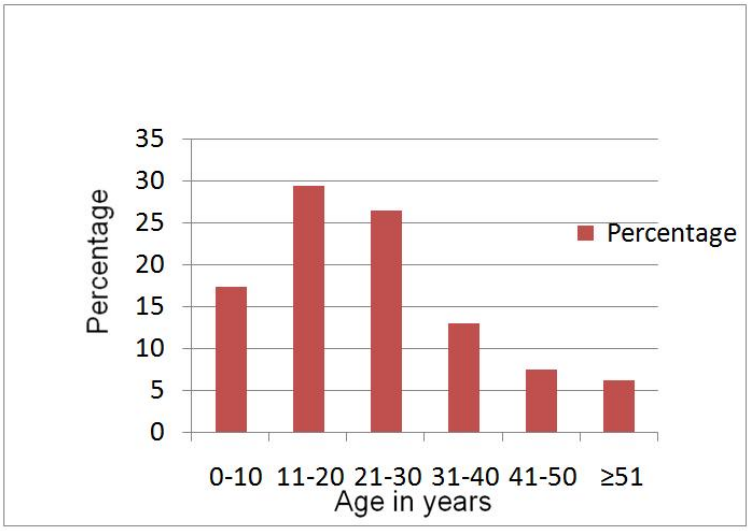

Chart 2. Age-wise distribution of the dengue and chikungunya IgM positve cases

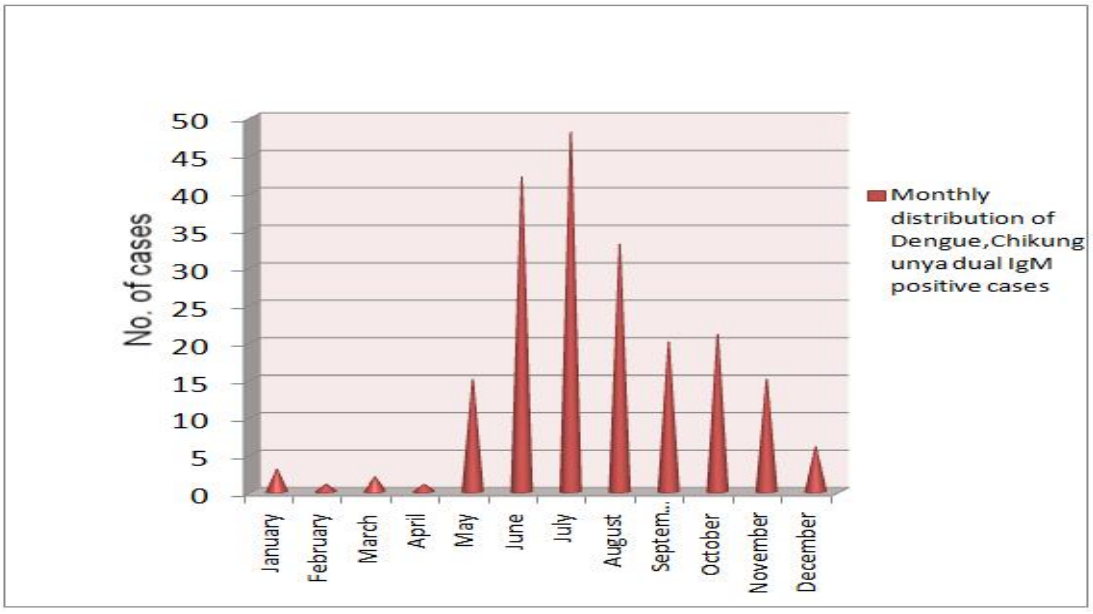

Chart 3. Monthly distribution of Dengue,Chikungunya dual IgM positive cases

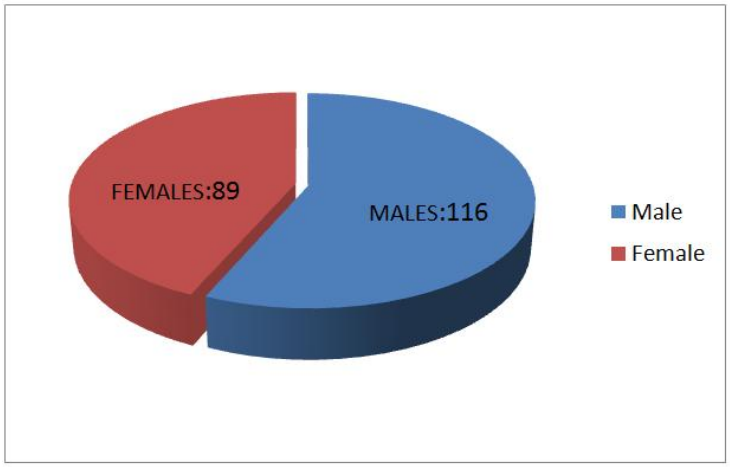

Chart 4. Sex distribution of dual IgM postive cases

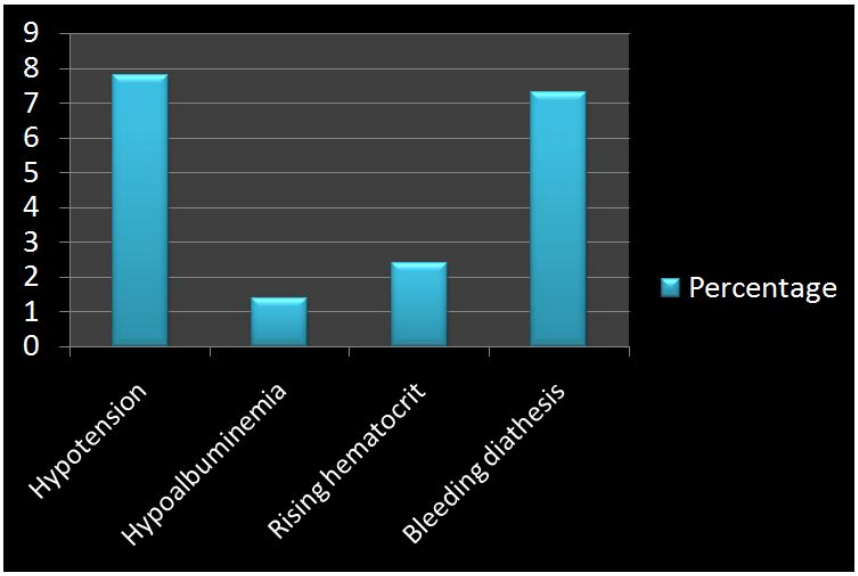

Chart 5. Percentage of Complications of Dengue

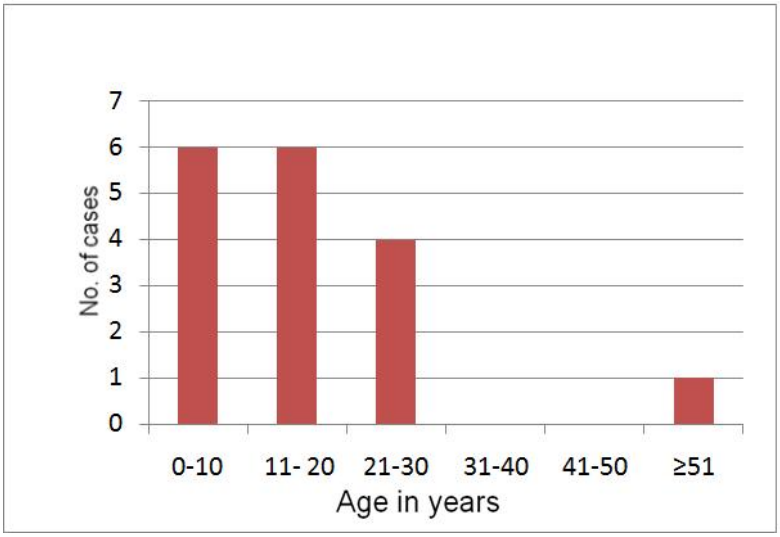

Chart 6. Age distribution of dengue complications

It indicates that concurrent infections may result in illness with overlapping signs and symptoms, making diagnosis and treatment difficult for physicians. Thus, in clinically suspected cases of Dengue or Chikungunya fever, it is advisable to test for both viruses especially in areas where they cocirculate. In our study, co-infection with Dengue and Chikungunya was found in 7.6% cases as compared to 2.7% in a study by Kalawat *et al*. (2011). The number of cases was more in the months of June to August and less during the months of January to April. This type of seasonal variation was seen in most of the studies, because [1] transmission intensifies at the start of the rainy season, when infected vector mosquitoes are more abundant as higher humidity lengthens their life span and increased temperatures shorten the extrinsic incubation period. The age group 11-20 years was mostly affected whereas lower number of cases were seen from elderly persons >51 years in our study. In the gender distribution, the number of affected males was more than females. These findings were much similar to the pattern shown by Kalawat *et al*. (2011). Where it was found that males were more frequently affected than females. In our case, co-infection with dengue and CHIK was found to in 4.77% cases as compared to 2.7% in a study by Kalawat *et al*. (2011).

Conclusion

With the urbanization that is occurring in India, the incidence of dengue infection is increasing dramatically. With the expectation that cases of co infection with DENV and CHIKV will become more prevalent in the future due to increased transmission of both viruses in various areas of India, enhanced surveillance to clinically and diagnostically differentiate CHIKV and DENV infections is needed for early recognition of virus invasion and local transmission, better patient care, and timely control measures. With clinical examination of CHIKV/DENV coinfected patients has not yet allowed the identification of specific or severe symptoms, such observations should be interpreted with caution. Our findings

may add to the recognition of CHIKV/DENV coinfections and suggest that tests to detect the presence of both viruses should be carried out in individuals showing clinical signs of an infection with either CHIKV or DENV.

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